ABSTRACT

MECHANISM OF SOIL FUNGISTASIS

by Wen-hsiung Ko

For 18 out of 22 fungi tested, the ability of their spores to germinate on soil was directly correlated ($r = 0.94$; $p < 0.5\%$) with the ability to germinate in the absence of exogenous nutrients. Fungi which required exogenous nutrients for germination failed to germinate on soil, and nutritionally independent fungi germinated on soil. Four exceptional fungi were nutritionally independent but did not germinate on soil. Their sensitivity to soil fungistasis was considered due to a strong diffusion gradient imposed by microbial activity in soil, by which nutrients diffused rapidly away from spores to soil. Evidence for this mechanism was the failure of these spores to germinate when leached with dripping sterile water. Nutrients were detected in the leach. Conversely, ascospores of Neurospora tetrasperma which germinated on soil also germinated during leaching with water. In addition, when nutritionally dependent spores were supplied with nutrients and placed on Millipore filters on soil, the nutrients were rapidly lost and the spores failed to germinate.

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Spores requiring exogenous nutrients for germination failed to germinate in aqueous extracts from natural soil, but they germinated in extracts from sterilized soil. Sterilization of natural soil by autoclaving increased free carbohydrates 27-fold and amino acids 37-fold. Based on a comparison of synthetic soil extracts with natural soil extracts, it was shown that extracts of sterilized soil contained sufficient nutrients for germination; extracts of natural soil did not.

In tests on alfalfa-supplemented natural soil, germination of Aspergillus fumigatus conidia occurred only in close proximity to the added organic matter, and not farther away. Extracts from alfalfa-supplemented natural soil, however, supported spore germination, whereas those of nonsupplemented soil did not. These experiments indicate that the bulk of soil apart from fresh pieces of organic matter is deficient in nutrients.

Decreased spore germination on agar disks incubated on soil was correlated with the rapid diffusion of nutrients from agar disks to soil. A similar decreased germination also occurred on water agar disks incubated with sterilized charcoal or washed with sterile glass distilled water.

The results indicate that soil fungistasis is a consequence of the unavailability in soil, or loss from spores, of nutrients required for spore germination.

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INTRODUCTION

Two striking properties of natural soil as it affects fungal behavior are that it prevents spore germination of most fungal species, and that it causes lysis of fungal mycelia if germination occurs or when mycelia are added to soil. Soil fungistasis is apparently of survival value in preventing lysis of vegetative hyphae under starvation condition. The purpose of this study was to investigate the mechanism of the widespread fungistatic effect of natural soil. The background information and techniques of this thesis are based on previous work done in this University (24, 26, 27, 28, 29, 30).

Antibiotics in soil were first proposed as the reason for failure of fungal spores to germinate in soil (28), during the pioneer work on this subject in this University. The failure to extract inhibitory substances from soil despite many attempts (24) led to a consideration of other alternatives. It was found that enhanced microbial activity occurred very rapidly after spores were placed in soil, and that this was due to exudation of nutrients from the spores (24, 27). It was further shown that germination of conidia of Glomerella cingulata and Helminthosporium victoriae in water was inhibited by washed cells of a variety of bacteria or streptomycetes (27). These results

suggested that soil fungistasis was a localized effect of enhanced soil microfloral activity in the spore vicinity. This enhanced activity might restrict spore germination in soil either by antibiotic production in the spore vicinity (24, 27), or by depletion of spore nutrients (27). Recently, in an extensive review of the literature on soil fungistasis, Lockwood considered competition for nutrients as the most promising explanation for inability of spores to germinate in natural soil (29).

This report provides evidence supporting the hypothesis that unavailability of nutrients required for spore germination is responsible for soil fungistasis. The necessity of exogenous nutrients for spore germination of various fungi was studied. Model systems were designed whereby fungistasis was reproduced without the presence of microorganisms or soil. The nutrient status of soil with or without added plant residues was studied.

LITERATURE REVIEW

Spores of most fungal species remain ungerminated in natural soil except in the vicinity of undecomposed organic matter or in the rhizosphere (29). The widespread occurrence of this inhibition was first established by Dobbs and Hinson (16) and has been termed fungistasis or mycostasis (11). The literature relating to soil fungistasis has recently been reviewed by Lockwood (29). This review will only include the literature dealing with the nature of fungistasis and important papers related to the subject which have appeared since Lockwood's review.

Many mechanisms have been suggested to account for the nature of the inhibition of fungal spore germination in soil. None, however, has received good experimental support.

Physical factors such as pH and redox potential seem to have been ruled out as causes of fungistasis. Agar made fungistatic by incubation with soil revealed no significant difference in oxidation-reduction potential from that of control agar which supported germination (23, 24). Moreover, autoclaving removed soil fungistasis but only slightly altered redox potentials (24). Although the pH of most soils is within the range tolerated by fungi (29), Green and his co-workers (18, 19) have suggested that pH might play a

part in soil fungistasis with certain fungi. Their test fungus, Trichoderma viride, germinated poorly outside the pH range 3.5-5.2 in tests made on agar. In their experiments, spore suspensions were adjusted with 0.1 N HCl to various pH values. Spores were then placed on disks of water agar. Tests were not done in solution or directly on soil. The effect of pH on spore germination might have been due to the release of soluble carbohydrates due to acid hydrolysis of the polysaccharides present in agar.

Most workers support the view that diffusible inhibitory substances are the cause of fungistasis. However, the nature and source of the postulated fungistatic substances in soil are viewed differently by different workers. Accumulation of carbon dioxide in soil is a possibility. But, soil fungistasis has been demonstrated under conditions of good aeration. Besides, amendment of soil with organic matter reduces fungistasis, yet it increases CO_2 production in soil (4).

Lingappa and Lockwood (25) once suggested that toxic lignin decomposition products might cause soil fungistasis. Since fungistasis occurs in soils low in organic matter, and the effect of lignin residues was distortion and inhibition of germ tubes rather than inhibition of germination, they later doubted that this is the case (29).

The possibility that a volatile inhibitor may be present has been suggested (11, 42), but Lingappa and

Lockwood (24) failed to obtain any evidence for this. Antibiotic substances in soil were suspected by Lockwood (28) and Weltzien (45), but germination in soil occurs in locations where antibiotic production is most likely to occur. If antibiotic production in natural soil occurs at all, the sites of production are most likely rhizospheres or in the vicinity of organic residues. However, these are precisely where germination occurs in soil. Furthermore, antibiotics in soil are subjected to physical inactivation and biological degradation. Enhanced microbial activity in the spore vicinity was shown by Lockwood and Lingappa (24, 27). They suggested that this might cause the inhibition of spore germination by virtue of antibiotic production (24, 27), or nutrient competition (27). Lockwood (29, 30) recently has cautioned that it is difficult to account for long-term inhibition with the hypothesis of antibiotic production since the spore nutrients which would be required to support the microbial production of antibiotics would eventually be depleted.

An analogy between the condition of fungi in soil and in staled fungal cultures has been proposed by Park (35, 36), and adopted by Griffin (20). Park (36) found that production of conidia and chlamydospores followed the cessation of mycelial growth and that the inhibition of spore germination and autolysis of mycelia also occured later in the same culture. This sequence was attributed to an

increasing concentration of a staling factor produced by the fungus in the medium.

A common staling substance produced by several unrelated fungi has been shown to exert inhibitory and possibly morphogenetic effects on mycelia of various fungi tested (36, 37, 38, 39, 41). Depletion of nutrients as the cause of the staling effect, an argument raised by Brian (4) and Lockwood (29), has been adequately ruled out (38, 39). Vacuolation of mycelium has been used as the index of the staling effect (39, 41). A similar vacuolation was induced by soil extracts (39), but pond-water, hypertonic solutions or toxic stains also showed the same effect. The staling substance was only slightly inhibitory or even had no effect on germination. Therefore, soil fungistasis is not likely to be due to staling substances in soil.

Dobbs and his co-workers believe that a soluble, very unstable inhibitor of microbial origin is the cause of soil fungistasis (12, 14, 15, 16, 17). Because of the competitive relationship with glucose and citrate they also believe that the inhibitor may be an antimetabolite (10, 17). Jackson (21), on the other hand, pointed out that if the postulated substance is of microbial origin, it must be resistant to chemical and biological inactivation in the soil, since the fungistatic effect persisted in fallow soil after being subjected to repeated intense leaching for

nearly two years. He viewed the inhibitors as complex organic substances, probably of microbial origin (22).

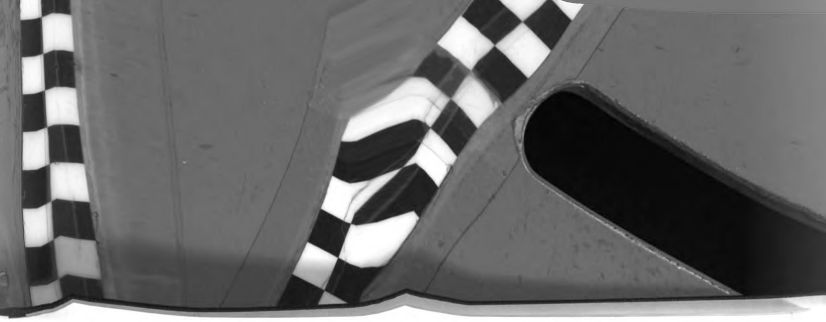
A thermostable inhibition of spore germination has been reported to occur in certain dune sands in Britain (13). This was termed residual mycostasis to distinguish it from the common thermolabile microbial mycostasis. It was suggested that $CaCO_3$ and iron were responsible for the residual mycostasis. However, since $CaCO_3$ and iron are very insoluble in water, inability of agar disks to support spore germination after incubation with dune sand is unlikely to be due to diffusion of such substances from sand to agar disks.

Many efforts have been made to extract fungistatic substances of whatever nature from soil, but the attempts have usually been unsuccessful, or the results, inconclusive (29). Many soil extracts were stimulatory instead of being inhibitory. Some nonsterile soil extracts have been reported to be inhibitory to mycelial growth or spore germination. But, in most cases the inhibitory effect disappeared partly or completely after sterilization by heat or filtration. Sterile inhibitory extracts have been obtained from a few soils (10, 43, 44). However, none of them have been shown to fulfill the characteristics of soil fungistasis, i.e., strong inhibition, widespread occurence and broad inhibitory spectrum. The fungistatic substances have never been characterized, concentrated, nor detected by any method other than biological assay.

Electrophoretic migration of an inhibitory effect in agar made fungistatic by incubation with soil (45), as well as induction of inhibition in agar disks by incubation with soil at low temperature (20), have been given as indirect evidence for the presence of fungistatic substances in soil. However, these can also be explained as the results of migration of soluble nutrients in agar or diffusion of nutrients from agar to soil.

Since the discovery of the widespread soil fungistasis, its interpretation on the basis of nutrients required for germination has usually been ruled out by the assumption that spores of test fungi are able to germinate in water (16, 24, 35). However, considerable data show that spores of many fungi require exogenous nutrients for germination (2, 8). That clay and some other inert materials prevented germination when spores were placed directly on these (24) is also possible supporting evidence for this interpretation. The fact that soil extracts often support spore germination is often taken as an argument that soil contains sufficient nutrients for germination. However, isolated nutrient sources within a deficient soil mass may provide enough nutrients to support germination in soil extracts.

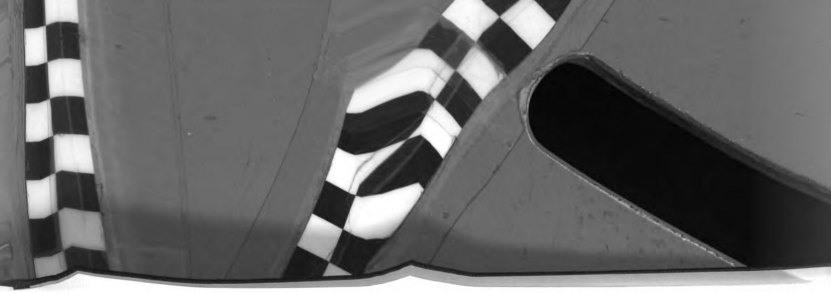
Cook and Schroth have suggested that competition for available substances in soil and elaboration of fungistatic substances by microflora are equally important to inhibition



of spore germination (9). Green and his co-workers (18, 19) believed that lack of nutrients essential for germination and soil reaction are responsible for soil fungistasis. However, the possibility that unavailability of nutrients alone is sufficient to account for failure of fungal spores to germinate in natural soil has not been ruled out.

MATERIALS AND METHODS

Preparation of fungal spores.--Conidia of powdery mildews and urediospores of rusts were kindly supplied by Dr. A. H. Ellingboe. Erysiphe graminis DC. f. sp. tritici Em. Marchal and Puccinia graminis Pers. f. sp. tritici Eriks. & H. Henn. were maintained on wheat (Triticum compactum Host 'Little Club'). E. graminis DC. f. sp. hordei Em. Marchal and P. coronata Cda. were maintained on barley (Hordeum vulgare L. 'Manchuria') and oats (Avena sativa L. ('Gopher'), respectively. Teliospores of Ustilago tritici (Pers.) Rostr., U. maydis (DC.) Cada., and U. nuda (Jens.) Rostr. were collected in Michigan during the growing seasons of 1964 and 1965. The following spores were obtained from cultures on potato-dextrose agar: conidia of Thielaviopsis basicola (Berk. & Br.) Ferr., Trichoderma viride Fr., Mucor ramannianus Möller, Penicillium frequentans Westling and Glomerella cingulata (Ston,) Spauld. & Schrenk; macroconidia of Fusarium solani (Mart.) Appel & Wr. f. phaseoli (Burk) Snyd. & Hans. and F. solani f. pisi (F. R. Jones) Snyd. & Hans. One isolate of M. ramannianus was kindly supplied by Dr. C. G. Dobbs. Conidia of Helminthosporium victoriae, Meehan & Murphy, Botrytis cinerea Pers. ex. Fr., and Aspergillus fumigatus Fresenius



were produced on V-8 juice agar (per liter: 200 ml. V-8 juice, 2 g. $CaCO_3$, 20 g. agar). Helminthosporium sativum Pam., King, & Bakke isolates #10 and #58 were kindly sent by Dr. R. D. Tinline, and were maintained on sterilized wheat straws in test tubes. Conidia as well as ascospores of Neurospora tetrasperma Shear & Dodge were collected from cultures grown on yeast maltose agar (per liter: 10 g. maltose, 4 g. yeast extract, 4 g. dextrose, 20 g. agar). Ascospores were separated from conidia by allowing them to sediment several times through a column of distilled water in a 100 ml. glass cylinder, and were stored under water at 4°C. until use.

For germination tests, conidia of mildews were shaken directly onto the medium to be tested. Urediospores of rust and conidia of N. tetrasperma shaken from plants or agar cultures, respectively, were suspended in water and used without washing. Conidia of the other fungi and the smut teliospores were washed 3 times with sterile glass distilled water in order to remove nutrients from culture media and plant hosts. For determination of percentage germination, 200 spores were counted for each treatment. All experiments were done at least twice.

Direct assay for fungistasis.--The method of Lingappa and Lockwood (26) was followed. Conover loam soil was sifted and stored in closed glass jars. The soil was moistened by damp paper towels placed on top of glass wool over soil until about 25% moisture was reached. Approximately

20 g. of such soil was placed on a small petri dish (50 x 15 mm.) and a smooth surface was made with a stainless steel spatula. Three drops (ca. 0.1 ml.) spore suspension were supplied onto the smoothed soil surface. After incubation the spores were stained with phenolic rose bengal and recovered with a solution of polystyrene. The dried plastic film was transferred to a drop of mineral oil on a glass slide, covered with a cover glass, and then observed microscopically.

Indirect assay for fungistasis.--Agar disks were prepared as follows: About 10 ml. of 2% Bacto agar (Difco) was poured into a petri dish (90 x 15 mm.) to provide a level surface. Another 15 ml. was pipetted onto the solidified agar. Disks 17 mm. in diam. and 2 mm. thick were cut with a cork-borer. Each disk had a volume of 0.47 ml. A soil block (about 70 x 6 mm.) with a smoothed surface was carefully transferred into a sterile petri dish to avoid contact with the edge of petri dish. The soil surface was covered with a sterilized disk of washed, noncoated cellophane (110 mm. diam.). The agar disks were placed onto the cellophane. After incubation one agar disk was transferred and incubated with nutrient agar to test the sterility of the disks.

Determination of carbohydrates.--Carbohydrates were determined by the method of Morries (33). The anthrone reagent was made by dissolving 0.2 g. of anthrone in 100 ml. of 7 M sulfuric acid (prepared by addition of 500 ml.

concentrated sulfuric acid to 200 ml. water). One ml. of the solution to be assayed was mixed in a boiling water bath for 10 minutes. The solutions were cooled and the optical density read at 600 mμ. in a colorimeter. A standard curve was made by measuring 1 ml. of samples containing 20, 40, 80 ug. of glucose.

Determination of amino acids and related compounds.-- Amino acids were determined according to the method of Moore and Stein (32). Ninhydrin reagent was prepared by dissolving 0.2 g. ninhydrin and 0.03 g. hydrindantin in 7.5 ml. methyl cellosolve, followed by addition of 2.5 ml. 4 N sodium acetate buffer (pH 5.5). To prepare 4N acetate buffer, 54.5 g. NaOAc. $3H_2O$ was dissolved in 40 ml. distilled water. Ten ml. glacial acetic acid was added and the solution was then made up to 100 ml.. One ml. of ninhydrin reagent was mixed in test tubes containing 1 ml. samples, and heated in a boiling water bath for 15 minutes. The solutions were then diluted with 8 ml. of 50% ethyl alcohol, and the optical density read at 570 mμ. in a colorimeter. A standard curve was made by measuring 4, 8 and 16 μg. of glycine in 1 ml. water.

RESULTS

Relation of nutrient requirements for fungal spore germination to soil fungistasis.--Explanation of soil fungistasis in terms of lack of nutrients required for spore germination implies that only those fungi which require exogenous nutrients for germination are sensitive to fungistasis. To test this possibility, the relation between spore germination on soil and in glass distilled water (a nutrient free medium) was studied among various fungi. For all the fungal species tested, spores were germinated directly on soil. Except for conidia of mildews, urediospores of rusts and ascospores of N. tetrasperma, germination of fungi in water was tested at three serial 10-fold dilutions to avoid inhibition from overcrowding. The depth of water in the small petri dish was limited to 0.5-1.0 mm. to provide good aeration. Mildew conidia were incubated in a moist chamber at 18°C. for 1 hour, followed by 10 hours at 22°C. (34). All other fungi were incubated at 24-28°C. There was a significant correlation ($r = 0.94$; $p < 0.5\%$) between germination on soil and in water when 18 of the 22 fungi tested were analyzed (Fig. 1, Table 1). Those spores which were able to germinate in water also germinated on soil, while those which were not able to germinate in water also failed to germinate on soil. All

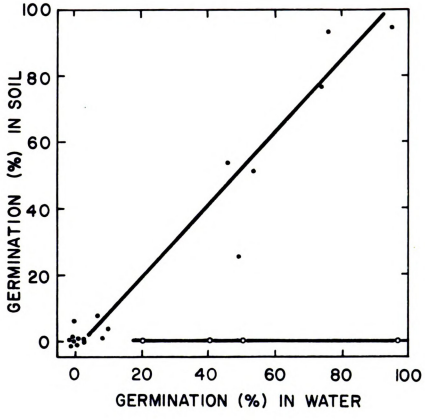


Fig. 1--Relation between fungal spore germination on soil and in water. Closed circles indicate 18 fungi for which a significant correlation ($r = 0.94$; $P < 0.5\%$) existed between germination on soil and in water. Open circles indicate 4 exceptions. Each point represents a different fungus.

Table 1.--Relation between spore germination on natural soil and in glass distilled water.

Fungus	Type	Germination(%)[a]	
		Natural Soil	Distilled Water
Neurospora tetrasperma	Ascospores	94	95
Puccinia coronata	Urediospores	93	76
P. graminis f. sp. tritici	Urediospores	53	46
Erysiphe graminis f. sp. tritici	Conidia	76	74
E. graminis f. sp. hordei	Conidia	51	54
Helminthosporium sativum #58	Conidia	25	49
H. sativum #10	Conidia	1	8
Fusarium solani f. phaseoli	Macroconidia	8	7
F. solani f. pisi	Macroconidia	6	0
Ustilago nuda	Teliospores	4	10
U. maydis	Teliospores	0	0
U. tritici	Teliospores	0	0
Trichoderma viride	Conidia	0	0
Penicillium frequentans	Conidia	0	1
Aspergillus fumigatus	Conidia	0	0
Mucor ramannianus	Conidia	0	0
M. ramannianus	Conidia	0	0
Botrytis cinerea	Conidia	0	1
Thielaviopsis basicola	Conidia	0	20
N. tetrasperma	Conidia	0	40
H. victoriae	Conidia	0	50
Glomerella cingulata	Conidia	0	96

[a]Data are from one of two experiments with similar results. Two hundred spores were counted in each treatment for each experiment.

test fungi which were not able to germinate in water germinated on nutrient agar. H. victoriae isolate #58 which possessed "inherent germinability" (6, 7), a term proposed to indicate the ability of spores to germinate in natural soil, germinated better in water than isolate #10 which did not possess such ability. Hence, sensitivity of spores to soil fungistasis seems to be restricted to those species which depend on exogenous nutrients for germination.

Conidia of G. cingulate, H. victoriae, N. tetrasperma and T. basicola were exceptional in that they germinated completely or partially in water but not on soil. Previous work in this laboratory showed that soil microorganisms rapidly utilized exudates from fungal spores including some of this type. Moreover, incubation of spores with soil bacteria and streptomycetes prevented germination of G. cingulata and H. victoriae in water without production of any detectable inhibitory substance (27). These results suggested that microbial acitivity in the immediate vicinity of fungal spores may result in loss of nutrients from the spores. To test this possibility, a system which provides for more efficient removal of nutrients from spores than occurs in water alone was designed.

Conidia of the 4 exceptional species were placed onto a Millipore filter (0.22 μ.) in a fritted glass filter holder. For comparison, conidia of A. fumigatus and P. frequentans

(which are nutritionally dependent) and ascospores of N. tetrasperma (which are nutritionally independent and germinate in soil) were also tested. Since conidia of G. cingulata germinated on cellophane or Millipore filters over soil, they were placed directly onto a fritted glass filter funnel (UF), instead. The spores were leached from 6-24 hours, according to the time required for germination, by dripping glass distilled water or 0.01 M phosphate buffer (pH 6.9) at the rate of 10-30 ml. per hour from a separatory funnel. Excess liquid on the filter was continuously removed by a small suction pressure (Fig. 2). By this means nutrients on spores were continuously carried away by water or buffer. The apparatus was sterilized before use and remained uncontaminated during the experiment. Spores on Millipore filters on soil were compared with those in the model system, except for G. cingulata which was placed directly on soil. For controls, spores were placed on wet Millipore filters in petri dishes or on the wet disc of a fritted glass filter funnel in the case of G. cingulata.

The nutritionally independent conidia of G. cingulata, H. victoriae, N. tetrasperma and T. basicola did not germinate under the leaching conditions nor on natural soil nor on Millipore filters on soil (Fig. 3). Ascospores of N. tetrasperma germinated in all the three test conditions, while conidia of A. fumigatus and P. frequentans failed to germinate.

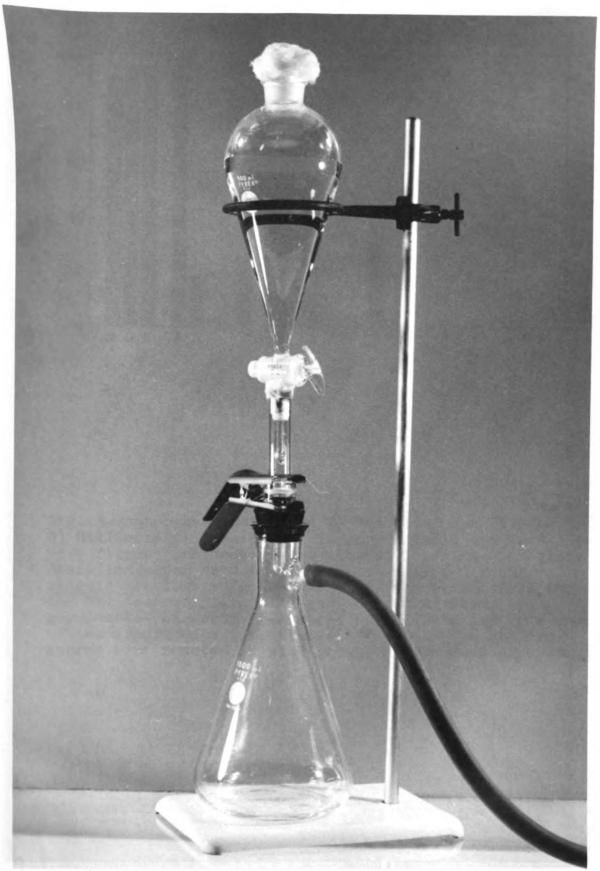


Fig. 2--The leaching system in operation. Spores were placed on a Millipore filter in the filter holder. Spores were leached by dripping glass distilled water or 0.01 M phosphate buffer (pH 6.9) at the rate of 10-30 ml per hour. Excess liquid on the filter was continuously removed by a small suction pressure.

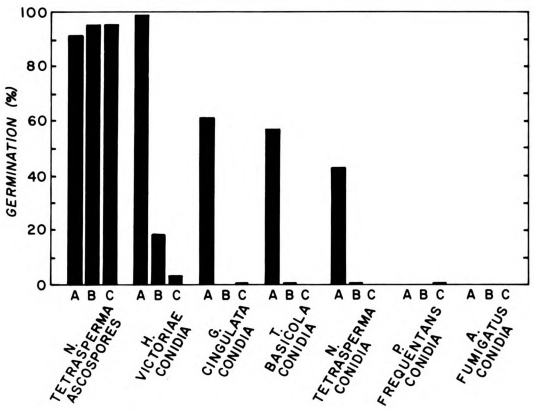
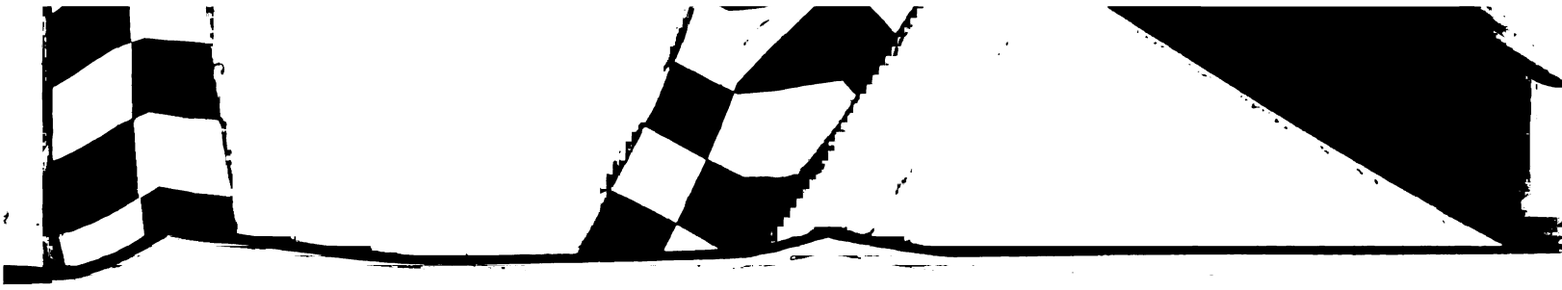


Fig. 3--Spore germination on A) wet Millipore filters, B) Millipore filters placed on natural soil and C) Millipore filters subjected to continuous leaching with glass distilled water or 0.01 M phosphate buffer. For G. cingulata a fritted glass disc (UF) was substituted for the Millipore filter in A) and C), and B) refers to germination directly on natural soil. Data are from one of two experiments with similar results. Two hundred spores were counted in each treatment for each experiment.



When leached conidia of H. victoriae and N. tetrasperma were transferred to a nutrient medium or when a 0.1% solution of glucose and peptone was allowed to drip on the spores, they germinated completely. It is, therefore, unlikely that the inhibition was caused by some injury to the spores. Since the germination response of each fungus when exposed to dripping water or buffer closely resembled the germination response of the same fungus exposed to soil itself, the model seems a valid approximation of the soil condition inducing fungistasis. Inability of the 4 exceptional nutritionally independent fungi to germinate in soil, therefore, seems related to the existence of a steep diffusion gradient from spores to soil.

To test whether continous exposure to a steep diffusion gradient is necessary for long-term inhibition of these nutritionally independent fungi, conidia of H. victoriae and N. tetrasperma were exposed to soil or leaching, then placed in water to germinate. A small Millipore filter (0.22 μ.; 25 mm. diam.) was placed on a larger one (0.22μ.; 47 mm. diam.) on soil and preincubated for 12 hours before spores were supplied. After 12 hours' incubation, spores were aseptically transferred to sterilized glass distilled water by dipping the small Millipore filter in water, and further incubated for 12 hours. For H. victoriae, treatments were also extended to 48 hours. Spores which had been exposed to soil germinated to nearly the same extent as those which had been leached. Three percent of the conidia of N.

tetrasperma and about 90% of those of H. victoriae germinated in water after being exposed to either soil or leaching. Apparently, for some fungi a short exposure to a strong diffusion gradient is sufficient to induce a nutritionally dependent condition which will result in long-term inhibition in soil, while for others, a longer or perhaps continuous exposure is necessary. This experiment again showed the similarity of natural soil and leaching system.

To determine whether nutrients were lost from leached spores, conidia (22 mg.) of N. tetrasperma were washed 3 times by centrifugation, then subjected to leaching on a Millipore filter. A total of 194 ml. leach was obtained after 12 hours leaching. The amounts of carbohydrates and amino acids were determined. A quantity of 6.9 mg. carbohydrates (glucose equivalent) and 8.2 mg. amino acids (glycine equivalent) per g. of spores were detected. Therefore, it seems likely that removal of nutrients is responsible for the failure of these spores to germinate under leaching conditions.

The ability of soil to induce nutrient loss from spores was tested further using nutritionally dependent spores. Conidia of A. fumigatus, P. frequentans, M. ramannianus were suspended in a nutrient solution containing 0.1% glucose and 0.1% peptone. Three drops (ca. 0.1 ml.) of the suspension were placed on Millipore filters on soil. After 12 hours incubation, spores were transferred to glass

distilled water or nutrient solution by immersing the Millipore filters in the liquids. Spores on moist Millipore filters were used as control. Conidia of all the three fungi germinated on Millipore filters, but failed to germinate when the filters were placed on soil (Table 2). After being transferred to water the nongerminated spores still did not germinate, but retained their ability to germinate in nutrient solution. The results suggest that if spores with sufficient nutrients to germinate are placed in soil, these nutrients are rapidly lost by diffusion into natural soil, and the spores fail to germinate.

Nutrient status of soil and its relation to fungistasis.--In view of the above results, the nutrient status of soil and its relation to soil fungistasis were investigated. It is well known that sterilization of soil by heat removes fungistasis (16). Since sterilization of soil by irradiation is milder than autoclaving, preserving for example enzyme activity (3, 31), fungistatic substances if present might not be lost in soil sterilized by this means. Soil was sterilized by gamma irradiation with a dosage of 4 megarads from a Co^{60} source, or by autoclaving for 40 minutes. Conidia of 5 test fungi: H. victoriae, H. sativum isolates #10 and #58, G. cingulata and P. frequentans, germinated completely on soil sterilized either by gamma irradiation or autoclaving. Therefore, removal of soil fungistasis by sterilization may be due to release of

Table 2.--Germination of spores carrying exogenous nutrients on Millipore filters placed on soil, and subsequent germination of the same spores in glass distilled water or a nutrient solution.

Treatment	Germination (%)[a]		
	M. ramannianus	*P. frequentans*	*A. fumigatus*
1. Millipore filters on soil[b]	0	0	0
2. Spores from (1) placed in distilled water	1	6	10
3. Spores from (1) placed in 0.1% glucose-peptone solution	99	98	70
4. Millipore filter control[b]	100	99	67

[a]Data are from one of two experiments with similar results. Two hundreds spores were counted in each treatment for each experiment.

[b]Spores were suspended in 0.1% glucose-peptone solution before being supplied to Millipore filters.

nutrients rather than to inactivation of fungistatic substances.

To test whether sterilization does release nutrients or not, the nutrient content in natural and sterilized soil were compared. Natural and autoclave-sterilized soils were shaken for 30 minutes with an equal amount of glass distilled water and centrifuged at 8,600 G. for 5 minutes. The

supernatant fluids were filtered through Millipore filters (0.22 μ.). The extract of natural soil was concentrated to about one-third of the original volume by evaporation at 50°C. The extract of natural soil contained 4.2 μg. carbohydrates (glucose equivalent) and 0.7 μg. amino acids (glycine equivalent) per ml., whereas the extract of sterilized soil contained 110 μg. carbohydrates and 19 μg. amino acids per ml. The pH range of the soil extracts was 6.7-7.0. Thus, natural soil contained 4.1 μg. carbohydrates and 0.5 μg. amino acids, while sterilized soil contained 108 μg. carbohydrates and 18.6 μg. amino acids per g. dry weight of soil. Sterilization of natural soil by autoclaving increased carbohydrates 27-fold and amino acids 37-fold. The results did not exclude the possibility of the presence of an inhibitor, since nutrients released by sterilization may overcome the effect of the inhibitor if the inhibitor is an antimetabolite. Consequently, spores were germinated in soil extract to detect the presence of the inhibitor.

Aqueous extracts from natural soil were sterilized by passage through a Millipore filter (0.22 μ.), and tested for germination. Conidia of A. fumigatus, P. frequentans and M. ramannianus failed to germinate in this extract. Since these fungi also did not germinate in water (Table 1), their failure to germinate in soil extract may be due either to the presence of an inhibitor or lack of nutrients in the extract. The soil extract did not support germination of the same three fungi even after the extract was autoclaved

for 40 minutes. Therefore, if there was an inhibitor, it must be a very heat stable one.

To base the inhibition on an inhibitor in the soil extract requires the demonstration that synthetic solutions containing the same amount of nutrients as extracts from natural and sterilized soil would permit greater germination in both cases. Synthetic soil extracts were prepared by addition of the same amounts of carbohydrates (glucose) and amino acids (peptone or casein hydrolysate) as the extracts from natural or sterilized soil to a mineral salt solution containing 0.25 g. K_2HPO_4, 0.08 g. NH_4NO_3, 0.25 g. $MgSO_4$, and 0.03 g. $FeCl_2$ in one liter glass distilled water. The synthetic soil extracts had the pH range of 6.9-7.1, and were autoclave-sterilized. Germination of F. solani f. phaseoli, A. fumigatus, P. frequentans and M. ramannianus in natural soil extracts was similar to germination of the same fungi in synthetic soil extracts (Fig. 4). When conidia of F. solani f. phaseoli were germinated in four 10-fold dilutions of natural and synthetic natural soil extracts, again germination in both extracts decreased similarly with increasing dilution of the solutions (Fig. 5). The results did not fulfill the requirements for presence of an inhibitor. On the contrary, all the experiments suggest that failure of spores to germinate in soil extract may be due to absence of sufficient nutrients for spore germination.

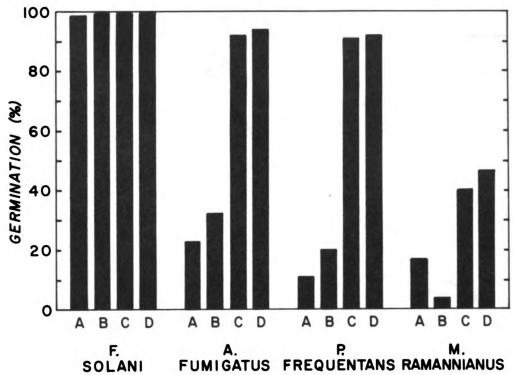


Fig. 4--Spore germination in A) aqueous extract from natural soil, B) synthetic extract of natural soil, C) extract of sterilized soil. Synthetic extract of natural soil contained mineral salts, 4.2 µg. glucose and 0.7 µg. casein hydrolysate per ml.; synthetic extract of sterilized soil contained mineral salts, 110 µg. glucose and 19 µg. casein hydrolysate per ml. Data are from one of the two experiments with similar results. Two hundred spores were counted in each treatment for each experiment.

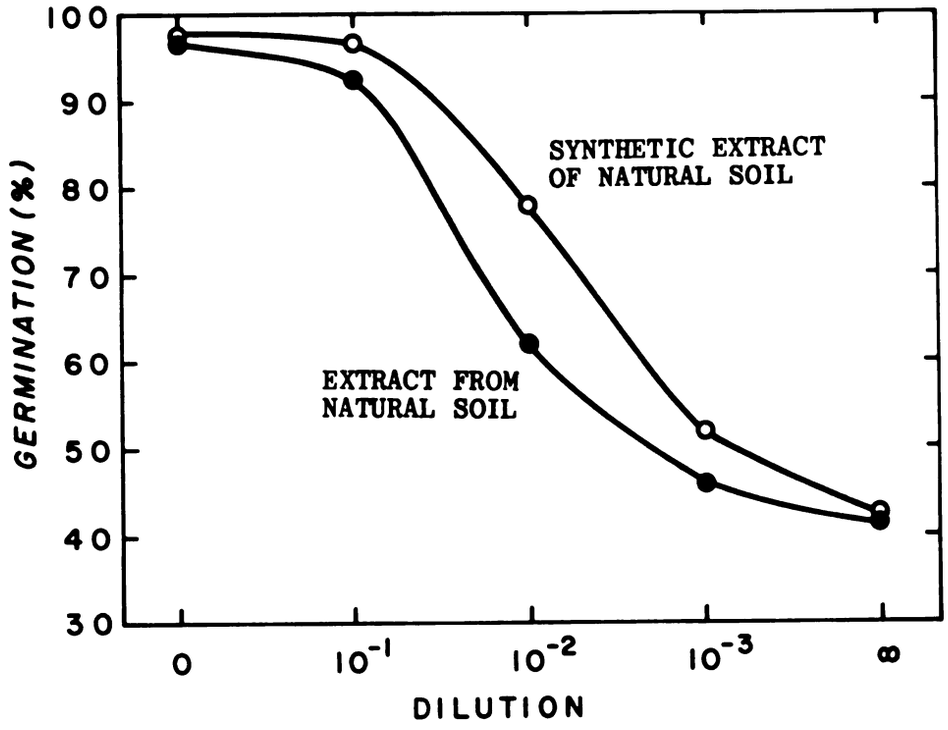


Fig. 5--Germination of macroconidia of F. solani f. phaseoli in a series of 10-fold dilutions of extract from natural soil and a synthetic extract of natural soil. Extract from natural soil contained 4.2 µg. carbohydrates and 0.7 µg. amino acids per ml. Synthetic extract contained mineral salts, 4.2 µg. glucose and 0.7 µg. casein hydrolysate per ml. Data are from one of two experiments with similar results. Two hundred spores were counted in each treatment for each experiment.

Annulment of soil fungistasis by nutrient amendment has been demonstrated in many cases (29). Since different fungi may require different nutrients for their germination, annulment of fungistasis in different fungi by different nutrients would be consistent with the view that the nutrients are acting to satisfy a nutrient requirement.

Natural soil was dried by blowing air from an electric fan and remoistened to about 25% moisture by addition of a solution containing 0.1% of various nutrients. Many kinds and combinations of nutrients were tried in order to find the minimum requirements for complete germination of each fungus on soil. Spores were germinated directly on the surface of the amended soils. Conidia of G. cingulata and N. tetrasperma required only glucose, while those of H. victoriae and P. frequentans required glucose and ascorbic acid for complete germination on soil. A. fumigatus required glucose, nicotinic acid and NH_4NO_3 (Table 3). Although amendment of natural soil with 0.1% glucose completely annuled fungistasis against conidia of G. cingulata and N. tetrasperma, addition of glucose at concentrations from 0.1 to 5.0% failed to promote germination of P. frequentans. The results then are consistent with the view that the nutrients were satisfying a requirement for germination rather than counteracting in some way an inhibitor.

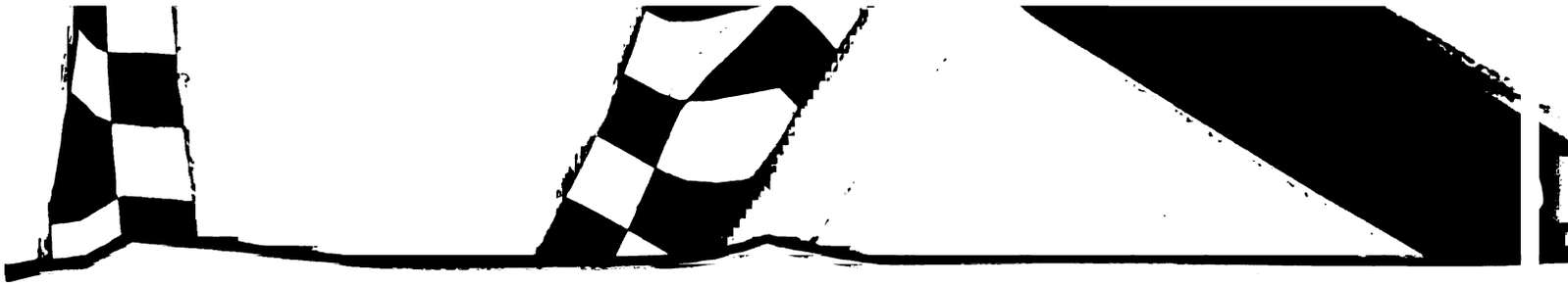
Table 3.--Nutrient requirements for conidial germination on natural soil supplemented with different nutrients.

Nutrients supplemented[b]	Germination (%)[a]				
	G. cingulata	N. tetrasperma	H. victoriae	P. frequentans	A. fumigatus
None	0	0	3	0	0
Glucose	98	97	47	0	0
Glucose + ascorbic acid	--	--	97	100	44
Glucose + nicotinic acid + NH_4NO_3	--	--	--	---	98

[a]Data are from one of two experiments with similar results. Two hundreds spores were counted in each treatment for each experiment.

[b]Soil was supplemented with 0.1% of each kind of nutrient.

The fact that extracts from natural soil often support germination must be explained if soil fungistasis is to be interpreted in terms of lack of nutrients in soil. Possibly, natural soil contains many isolated sources of nutrients, and fungistasis is expressed only apart from such nutrient sources. Water extracts of natural soil may, thereby, gather enough nutrients from the isolated nutrient sources to support germination. Since A. fumigatus did not germinate in extracts from natural soil, it was employed to test this possibility. Conidia of A. fumigatus should



germinate on soil near added plant residues but not away from them. A water extract from such soil would be expected to support conidial germination.

Twenty g. of Conover loam soil were placed in a small petri dish and compressed. Twenty mg. of finely chopped dry residues of mature barley or green alfalfa were placed in a small furrow made in the center of the soil surface, then covered with a thin layer of soil. Germination of A. fumigatus was tested direct on soil. Conidia germinated on the soil surface over the tope of the alfalfa residues, but failed to germinate at distances greater than about 1 mm. from them (Table 4). Residues of mature barley did not stimulate germination of A. fumigatus on soil. It seems likely that green alfalfa residues contain more soluble nutrients than do residues of mature barley. Soil with 0.1% barley or alfalfa residues were shaken with the same amount of glass distilled water for 30 minutes. The soil suspensions were centrifuged and the supernatants filtered through Millipore filters (0.22 μ.). The filtrates were sterilized by autoclaving and tested for germination. Extracts were also prepared from nonsupplemented natural soil. Germination of A. fumigatus in the extract from barley-amended or from non-amended soil was very poor, whereas germination in the extract from alfalfa-amended soil was nearly complete. The amounts of carbohydrates and amino acids in the extract from barley-amended soil was

Table 4.--Effect of added plant residues on germination of conidia of A. fumigatus on natural soil and in aqueous soil extracts.

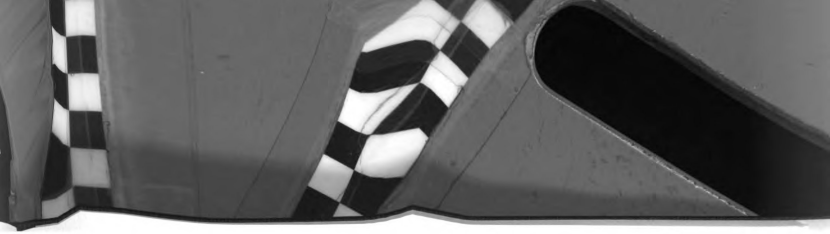
	Alfalfa-amended soil	Barley-amended soil	Non-amended soil
	Germination (%)[a]		
Near residue (< 1 mm)	85	0	0
Away from residue (> 1 mm)	0	0	0
Soil extract	88	22	28
	Nutrients (uG/ml)		
Carbohydrates[b]	52	6	4
Amino Acids[c]	3.4	0.7	0.3

[a]Data are from one of two experiments with similar results. Two hundred spores were counted in each treatment for each experiment.

[b]Glucose equivalent.

[c]Glycine equivalent.

very low and did not differ from these in plain soil extract (Table 4). The extract from alfalfa-amended soil, however, contained about 10 times more carbohydrates and amino acids than the other two extracts. Evidently, extraction can gather sufficient nutrients from organic particles in natural soil to support spore germination in the extracts.



Nutrient status of agar disks and its relation to fungistasis.--Inability of agar disks to support spore germination after incubation with soil has been interpreted as the result of diffusion of fungistatic substances from soil to agar disks (29). Like agar disks, moist filter papers became fungistatic after incubation with soil. This provided a more direct means to test whether a fungistatic substance had diffused into such materials incubated on soil. Ten Whatman No. 1 filter paper disks (7 cm. diam.) and 7 ml. distilled water were placed in a petri dish and autoclaved, then incubated with a soil block separated from the filter papers by a sheet of sterilized cellophane. After 9 days incubation at 1°C., the soil block was removed. The dishes were transferred to 24°C., and the cellophane was replaced with a new sheet. P. frequentans failed to germinate, and H. victoriae germinated poorly on the cellophane on these filter papers. However, when liquid pressed aspectically from such fungistatic filter papers was transferred to fresh filter papers and tested for germination, the same fungi germinated well. The failure of moist filter papers or agar incubated on soil to support spore germination is, therefore, unlikely to be due to the diffusion of fungistatic substances from soil.

The alternative possibility that inhibition resulted from loss of nutrients by diffusion to soil was investigated. Attempts were made to induce a fungistatic effect in agar disks by methods which would withdraw nutrients from the agar.

Water agar disks were incubated with charcoal or washed with water. Thirty grams charcoal (Norit A, decolorizing carbon) were mixed with 400 ml. ethyl alcohol and filtered through a Whatman No. 1 filter paper to remove possible imputities which might interfere with germination. The charcoal was then washed and filtered 3 times with a total of 1 liter of glass distilled water, and dried in an oven at 80°C. for 12 hours. A charcoal block was prepared as previously described for a soil block, then sterilized by autoclaving. Water agar disks were placed over a sheet of washed and sterilized cellophane on the charcoal or natural soil. After incubation for 12 hours at 24°C., agar disks were transferred to sterile petri dishes and tested for spore germination. To wash water agar disks, 10 disks were shaken or stirred with a magnetic stirring bar in 500 ml. sterilized glass distilled water for 12 hours, then transferred to petri dishes for germination tests. Disks without treatment were used as controls. Sterility of the treated agar disks was tested on nutrient agar.

Agar disks washed with distilled water or incubated with sterilized charcoal reporduced the fungistatic effect of agar disks incubated with soil (Fig. 6). Conidia of G. cingulata, P. frequentans, A. fumigatus, and M. ramannianus failed to germinate on agar disks incubated with soil or sterilized charcoal. Washed agar disks also failed to support germination of A. fumigatus and M. ramannianus

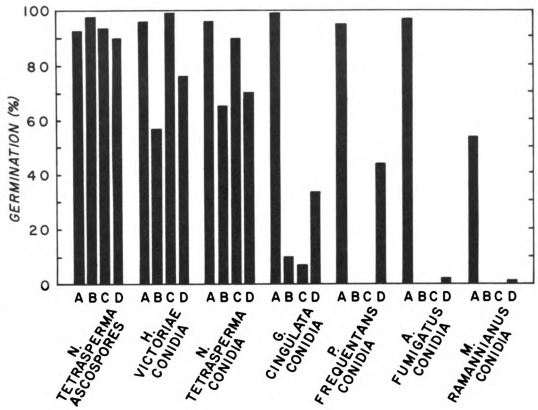
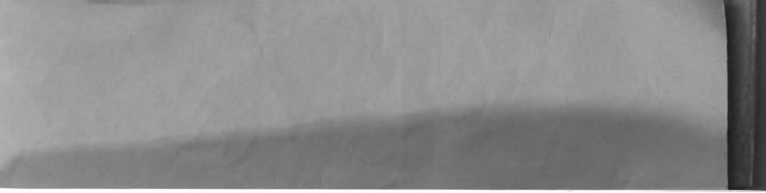


Fig. 6--Comparison of fungal spore germination on water agar disks transferred to sterile petri dishes after 12 hours treatment as follows: A) without treatment, B) preincubated on cellophane on natural soil, C) preincubated on cellophane on sterilized charcoal, and D) washed with glass distilled water. Data are from one of two experiments with similar results. Two hundred spores were counted in each treatment for each experiment.



and gave reduced germination of P. frequentans and G. cingulata. Germination of conidia of H. victoriae and N. tetrasperma was reduced on agar disks incubated with soil or washed with distilled water. Ascospores of N. tetrasperma germinated on all 3 treatments. Apparently leaching of nutrients from agar disks can render them fungistatic. Diffusion of nutrients from agar disks to soil may be responsible for failure of agar disks on soil to support germination.

The rate of diffusion of substances from agar disks to soil was tested by incubating agar disks supplemented with colored substances on soil. Water agar disks were immersed in red (2.5%), green (2.5%), blue (1.5%) food colors (pH 7.0) for 30 minutes. The colored agar disks were then placed on sterilized cellophane on natural soil, and incubated at 1° and 24°C. Disks from each temperature were removed after 2, 6, 12, and 24, hours incubation and the intensity of colors compared with control disks kept in sterile petri dishes. At 24°C., the intensity of color in agar disks decreased with time of incubation (Fig. 7). Within 2 hours a noticeable decrease in color intensity had occurred. At 1°C. the rate of decrease in color intensity was slower. The rapid diffusion of food colors from agar disks to soil suggested that soluble nutrients may diffuse rapidly from agar disks to soil.

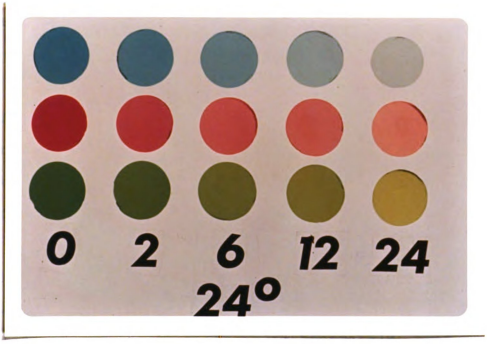
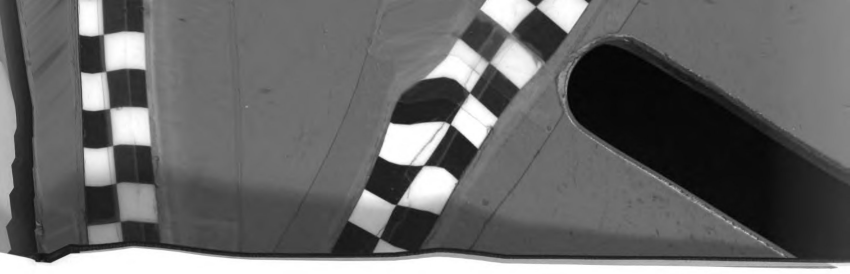


Fig. 7--Agar disks supplemented with vegetable food colors showing loss of colored material to soil by diffusion during incubation for 0-24 hours at 24°C. The experiment was done twice with similar results.



To determine the rate at which nutrients diffused from agar disks, 2% Bacto (Difco) water agar was supplemented with 1.7 g. glucose and 3.7 ml. casein hydrolysate (10%) per liter in combination or separately. The agar disks were placed on a sheet of sterilized cellophane on soil, and were removed at different time intervals during incubation at 1 or 24°C. Nutrients in the disks were extracted by shaking 3 disks in 6 ml. distilled water in a 50 ml. Erlenmeyer flask for 30 minutes. The extract was filtered through a Millipore filter (0.22 μ.). Rapid loss of nutrients from agar disks was revealed. Disks supplemented with nutrients, either singly or combined, lost 40-50% of the carbohydrates and amino acids within 6 hours at either 1 or 24°C. (Fig. 8, 9). When agar was supplemented with glucose or casein hydrolysate separately, both carbohydrates and amino acids continued to decrease after 12 hours (Fig. 9). When glucose and casein hydrolysate were combined, however, the amount of each decreased with time up to 12 hours incubation at either 1 or 24°C. (Fig. 8); after 12 hours, carbohydrates in the agar continued to decrease, but amino acids increased. The increase in amino acids in agar disks after 12 hours may be due to the exudation or release from living or dead microorganisms. Nutrients naturally occurring in plain water agar disks also were rapidly lost when disks were placed on soil. Carbohydrates decreased for 24 hours, but amino acids decreased first then increased again.

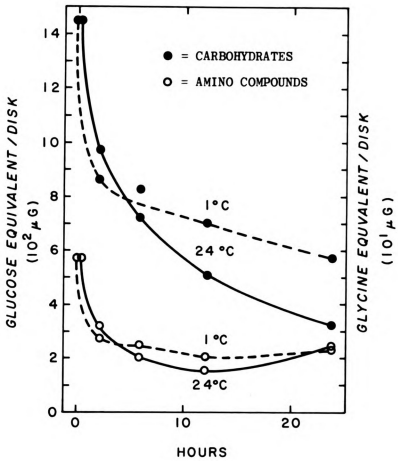


Fig. 8--Changes in amounts of carbohydrates and amino acids in agar disks amended with glucose and casein hydrolysate during incubation on natural soil at 1 or 24°C. Data are from one of two experiments with similar results. Two hundred spores were counted in each treatment for each experiment.

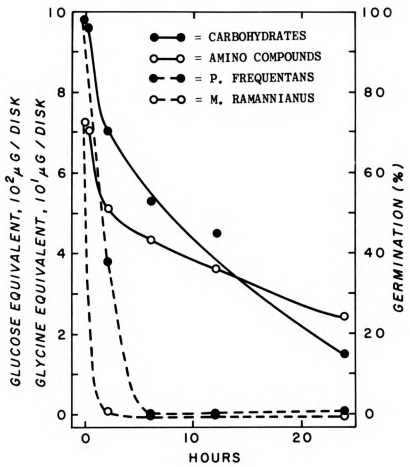
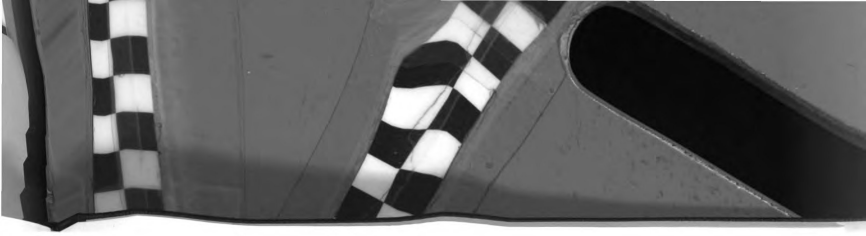


Fig. 9--Relation between loss of carbohydrates and amino acids from agar disks to soil during incubation at 24°C. and decrease in ability of agar disks to support germination. For nutrient analysis, agar disks were supplemented with glucose and casein hydrolysate. For germination tests, plain water agar disks were removed from soil at the same time intervals. Data are from one of two experiments with similar results. Two hundred spores were counted in each treatment for each experiment.



Plain water agar disks without added nutrients were incubated on soil for different time intervals to test spore germination. Germination decreased with time and was correlated with loss of nutrients from nutrient supplemented disks incubated with soil (Fig. 9). After 6 hours incubation with soil at 1 or 24°C., plain water agar disks no longer supported germination of P. frequentans nor M. ramannianus. The results further suggest that diffusion of nutrients from agar disks to soil may account for inability of such disks to support germination.

DISCUSSION

Inability of fungal spores to germinate in most natural soil could be due either to the presence of inhibitory substances or the absence of nutrients required for germination. Many attempts have been made to extract fungistatic substances from natural soil. Although a few workers (10, 43, 44) have obtained sterile inhibitory extracts from a few soils, most such attempts have been unsuccessful or the results inconclusive (29). The minimum requirements for the demonstration in soil extracts of an inhibitory substance which is responsible for soil fungistasis are the following: (i) the inhibitor must be extractable from a wide range of soil; (ii) it must be present in the same soil through the whole year so long as the soil is fungistatic; (iii) it must be inhibitory to a wide range of fungi; (iv) it must be a very potent inhibitor; (v) spores of those fungi which are not sensitive to soil fungistasis must not be inhibited. Thus far, none of the requirements have been met, even in the few cases where an inhibitor has been demonstrated.

There are several other reasons for doubting that the postualted inhibitors exist in natural soil. (i) Sterilization by autoclaving removed fungistasis in soil,

but did not alter the fungistatic effect in the soil extracts prepared by the method described here. (ii) Conidia of H. victoriae germinated better in water after being exposed to the influence of soil fungistasis. (iii) Spores germinated in liquid expressed from fungistatic moist filter papers. (iv) Different fungi required different nutrients for annulment of soil fungistasis. It is very unlikely that natural soil contains antimetabolites against glucose, ascorbic acid, nicotinic acid, NH_4NO_3 and other compounds. (v) Different propagules of the same fungus responded differently to soil fungistasis. Conidia of N. tetrasperma failed to germinate on soil, while ascospores of the same fungus germinated freely.

Several findings indicate strongly that for most fungi inability of spores to germinate on natural soil is due to lack of nutrients therein which are required for germination. Most of the fungi tested required exogenous nutrients for germination and all of these failed to germinate on soil (Table 1). Extracts from natural soil contained insufficient nutrients to support germination of spores of A. fumigatus, P. frequentans and M. ramannianus, and presumably this is also true for other nutritionally dependent fungi. Explanation of soil fungistasis in terms of nutrient requirements for germination is also supported by the germination on soil for most of those fungi which

were nutritionally independent, i.e., germinated in water alone, though there were some exceptions.

The failure of four of the nutritionally independent fungi to germinate on soil may be due to the existance of a steep and continuous diffusion gradient from spores to soil, resulting in greater loss of nutrients from spores than occurs in water. This possibility was previously indicated by the rapid utilization of nutrients from spores by microorganisms in soil, and by the inability of the nutritionally independent conidia of G. cingulata and H. victoriae to germinate in water during incubation of the washed spores with washed bacteria or streptomycetes (27). The experiments performed in the leaching system support the hypothesis that inhibition of these fungi occurred as a result of a diffusion gradient. Under sterile conditions, conidia of the 4 exceptional fungi, which germinated in water but not on soil, failed to germinate when exposed to dripping water. Analysis of the leachate from N. tetrasperma conidia showed that nutrients were lost from the spores by the irrigation treatment.

The existence of a strong diffusion gradient affecting nutrinets applied to soil is further indicated by the rapid diffusion of colored substanced and nutrients from agar disks to soil. Moreover, when nutritionally dependent spores carrying enough nutrients to germinate were placed on Millipore filters on soil, nutrients were lost and the spores failed to germinate.

Several lines of evidence suggest the similarity of the leaching system to natural soil. (i) Like natural soil, the leaching system also possesses the ability to cause lysis of fungal mycelia. Mycelia of H. victoriae subjected to leaching in the model system were lysed within 4 days (unpublished data). (ii) The pattern of spore germination in the model system completely correlated with that occurring on natural soil. The nutritionally dependent fungi as well as the 4 exceptional nutritionally independent ones failed to germinate both on soil and in the leaching system. On the other hand, ascospores of N. tetrasperma, which germinated on natural soil, also germinated freely in the leaching system. (iii) Ability of different fungi to germinate after being exposed to leaching was the same as that after exposure to natural soil. For example, conidia of H. victoriae germinated completely after exposure to soil or leaching system, while conidia of N. tetrasperma failed to germinated until exogenous nutrients were added.

Apparently, inability of N. tetrasperma conidia to germinate during leaching with water was due to depletion of nutrients, but this was not true for H. victoriae, at least up to 48 hours. Possibly conidia of Helminthosporium have developed the ability to respond to a strong diffusion gradient by changing the permeability of the cell membrane which results in preventing loss of nutrients from inside the conidia, or in some other way postpone germination.

This may be the means by which conidia of Helminthosporium are able to survive in soil for extended perios (5).

Chinn and his co-worker (6, 7) found that some isolates of H. sativum were able to germinate in soil though most were not. Genetic factors were shown to be involved in the ability of spores of this fungus to germinate in soil. They proposed that enzyme systems of spores of isolates able to germinate in natural soil were unaffected by fungistasis, or that these spores excreted substances capable of neutralizing fungistatic substances. However, my results suggest that isolates possessing "inherent germinability" are resistent to fungistasis because they contain a higher proportion of nutritionally independent spores. More spores of isolate #58 which possessed "inherent germinability" were able to germinate in water than those of isolate #10 which did not have the ability to germinate in soil.

In this report, extracts from natural soil have been shown to contain insufficient nutrients to support spore germination of P. frequentans, A. fumigatus, and M. ramannianus, but contained enough nutrients for germination of F. solani f. phaseoli. Extracts from fungistatic natural soil frequently have been reported to support spore germination, and this is often given as an argument that soil contains sufficient nutrients for spore germination (29). However, Alexander (1) has pointed out that nutrients are

not uniformly distributed throughout soil, but are compartmentalized in microsites. The kinds and amounts of available nutrients differ from site to site. Moreover, essential substances in natural soil may be bound in some way by clay. Leaching the soil may release the bound substances or gather different substances together so that requirements for spore germination may be met in the extracts. My results with alfalfa-amended soil show that natural soil containing organic residues is fungistatic apart from the residues, but that extracts from such soil can accumulate sufficient nutrients from the residues to support spore germination.

Cook and Schroth (9) reported that addition of antibiotics to soil increased germination of chlamydospores of F. solani f. phaseoli. They interpreted this increased germination as due to the combined effect of reduced competition for available nutrients in soil and reduced elaboration of fungitoxic materials. However, the results described here suggest that competition for nutrients alone may be sufficient to account for inability of fungal spores to germinate on natural soil. Recent results by Powelson (40) also suggest that diffusible carbon substrates are the major factor limiting fungal spore germination in soil. He failed to obtain germination of Verticillium dahliae conidia, which require an exogenous source of both carbon and nitrogen, in a silica gel medium (nutrient free)

containing natural soil, unless the medium was supplemented with glucose. Increased germination by addition of antibiotics to soil may be caused by a decrease in the diffusion gradient or by release of small amounts of nutrients through partial inactivation of microorganisms. Chlamydospores of Fusarium may resemble H. victoriae conidia which failed to germinate during leaching, but retained their ability to germinate when the diffusion gradient was reduced, or they may resemble conidia of F. solani which required only trace amounts of nutrients to trigger germination.

Indirect evidence for the presence of fungistatic substances in soil was given by Weltzien (45) and Griffin (20). Weltzien showed that the inhibitory effect in fungistatic agar migrated to the anode in gel-electrophoresis. However, his experiment could also be interpreted as the result of accumulation of electrolytes or migration of essential nutrients away from the anode. Griffin obtained inhibitory agar by placing water agar disks on cellophane on soil for 9 days at 2°C. to allow diffusion of material into agar from soil with minimum microbial activity. My results indicate that nutrients from agar will diffuse rapidly to soil under these conditions and that this may cause inhibition of spore germination. Liquid pressed from moistened filter papers made fungistatic by incubation with soil at 1°C. for 9 days also was not inhibitory to

spore germination. Both colored substances and nutrients in agar disks decreased rapidly with time of incubation on soil at 1°C. A concomitant decrease in ability of agar disks to support spore germination accompanied loss of nutrients. Moreover, water agar disks washed with water or incubated with sterile charcoal also failed to support spore germination.

The nutrient deficiency hypothesis permits a simple explanation for many aspects of soil fungistasis, such as the following: (i) its widespread occurrence existing in nearly all soil types assayed; (ii) its resistance to physical inactivation and biological degradation in soil; (iv) the restoration of fungistasis to sterilized soil by infestation with non-antibiotic producing microorganisms; (v) its non-lethal effect to spores; (vi) the annulment of fungistasis by nutrients (34); (vii) its occurrence in inert materials such as clays.

The results described herein suggest that soil fungistasis is a consequence of the unavailability in soil, or loss from spores, of nutrients required for spore germination. Therefore, there is no necessity of postulating the existence of fungistatic substances to account for failure of fungal spores to germinate on natural soil.

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